# THE TIME RELATIONS OF ACID PRODUCTION IN MUSCLE DURING CONTRACTION.

By H. E. ROAF.

*(From the Laboratory of Physiology, St Mary's Hospital Medical School.)*

FOR many years it has been known that muscular contraction causes a production of acid. By means of litmus paper du Bois Reymond demonstrated that resting muscle is alkaline whilst fatigued muscle is acid (1). Dreser (4) improved the demonstration by injecting acid fuchsin under the skin of a frog and by stimulating some of the muscles after twenty-four hours. The result of this experiment was that the resting muscles were colourless but the stimulated muscles became pink owing to the acid causing a change in colour of the indicator.

I have recently tried a similar experiment at the Port Erin Biological Station, showing the probable production of acid in a single contraction. Various indicators were dissolved in sea water and small medusæ placed in the solutions. The most successful experiment was in the case of neutral red, in which the medusæ stain a faint pink. At each contraction of the muscular plate the colour became a deeper red and then faded again. This change in depth of colour was too marked to correspond to the concentration due to the diminution in area of the umbrella during contraction; therefore the cause was probably the production of acid.

The importance of acid production during muscular contraction was emphasised by the work of Fletcher and Hopkins on lactic acid (5). They found that normal resting muscle contained very little or no lactic acid but that muscle which had been caused to contract contained appreciable amounts. The maximum amount was 0·19 p.c. for contraction and 0·33 p.c. for chloroform rigor. They also showed that after the contraction was finished the lactic acid was removed by some process which required the presence of oxygen. Their observations show that lactic acid is associated with muscle contraction but they do not prove that the acid is causative. Later work by A. V. Hill has shown that the amount of lactic acid produced is proportional to the tension developed in the muscle (7).

In view of the importance of this subject the experiments dealt with in this paper were undertaken and the acid production of muscle has been studied by means of electrodes in the hope that the time relations would show whether the acid appears early or late in the process of contraction. The results indicate that the formation of acid probably precedes the shortening.

An outline of the method has been previously given (10). It consists in placing two dissimilar electrodes on electrically symmetrical portions of the muscle (Fig. 1). One of these electrodes is a calomel electrode, *C*, consisting of mercury and calomel in contact with a Ringer solution of the same osmotic concentration as frog's blood and placed in contact with the muscle by a wick soaked in Ringer solution. The other electrode, *M*, is one that reacts to changes in the hydroxyl ion concentration. As the product of the concentration of hydrogen and hydroxyl ions is constant, a change in hydroxyl ion concentration is accompanied by the reciprocal change in the hydrogen ion concentration: therefore measurements of the hydroxyl ion concentration can serve to show the changes in the hydrogen ion concentration.

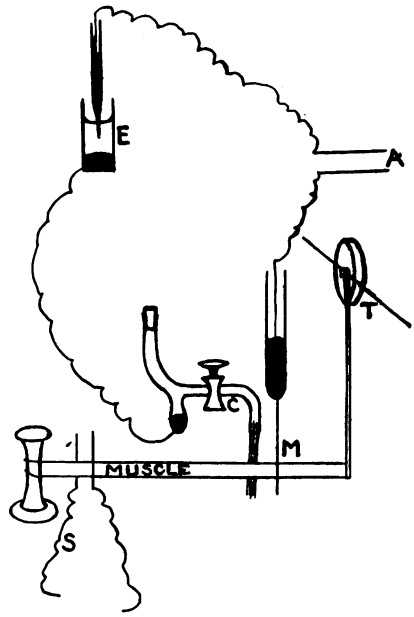


Fig. 1. Diagram representing the method of measuring the change in hydroxyl ion concentration of muscle. *S* stimulating electrodes, *T* tension lever, *C* calomel electrode, *M* manganese dioxide electrode, *E* capillary electrometer, *A* external compensating current.

The electrical potential produced by the contact of these two dissimilar electrodes with the muscle is balanced by an external current *A* and the muscle is caused to contract. As the electrodes are so arranged that two calomel electrodes on the same points at which the dissimilar electrodes are placed, showed no change of potential when the muscle contracts, any change in potential must be due to changes in concentration at the contacts of the electrodes with the muscle. The change of potential at the calomel electrode must be very slight as it is a contact between two liquids containing approximately

the same concentrations of electrolytes and a slight change of concentration would have very little effect. Therefore any appreciable change of potential must be at the contact of the hydroxyl ion electrode and the muscle.

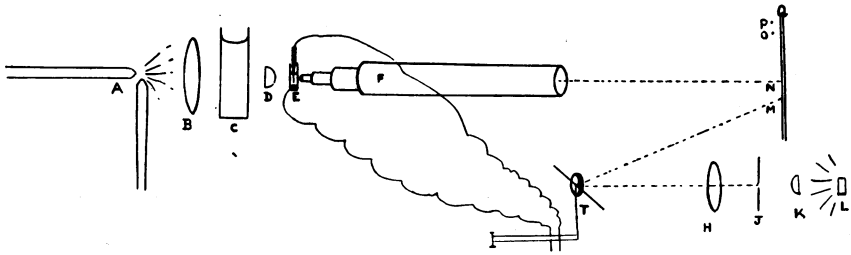


Fig. 2. Diagram showing arrangement of apparatus for recording electrical change and movement of muscle. *A* arc lamp, *B* condenser, *C* cooling trough, *D* bull's eye condenser, *E* capillary electrometer, *F* microscope, *N* level of mercury image on camera slit before contraction, *L* lime light, *K* condenser, *J* transverse slit, *H* lens, *T* tension lever with mirror, *M* image of slit *J* on mercury shadow on camera slit, *Q* camera slit, *O* signal in primary circuit of induction coil, *P* signal of time marker.

The hydroxyl ion electrode consists of manganese dioxide deposited electrolytically on platinum wire from a solution of a manganese salt (13). Unfortunately this electrode is said to be incorrect for quantitative measurements (12) but this does not interfere with its present use which is merely to measure the relation of the time at which the acid is produced to the contraction of the muscle.

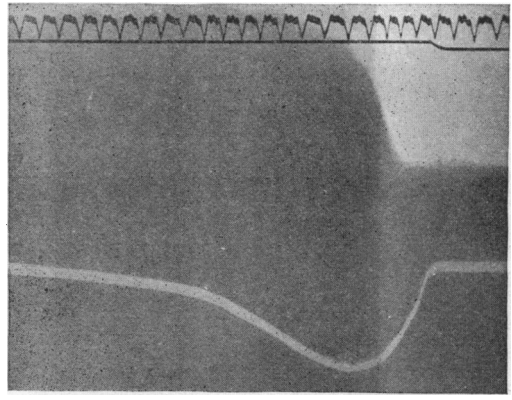

Fig. 3. Manganese dioxide electrode positive, compensated. Increase in positive charge at manganese dioxide electrode beginning 0·013″ after beginning of the shortening of muscle. Time marker 0·01″.

The main difficulty is the preparation of the electrodes. The best method seemed to be to attach the electrode to the positive pole of a five volt current through a wire resistance of ten ohms, the resistance of the liquids being unknown. The negative pole was attached to another platinum electrode and the current passed for about one minute. After use they may be recoated by electrolysing again for about half a minute. After several recoatings it is better to clean them by soaking them in concentrated sulphuric acid and to start afresh. After the first coating of the electrode the electrometer shows a greater change of potential (Fig. 3) than after the subsequent coatings (Fig. 4). This agrees with the statement of Tower that a thin coating reaches equilibrium sooner than a thick one. In the present experiments the alteration of acidity is so rapid that equilibrium is not attained, therefore a thin coating shows a greater change of potential as the electrode approaches nearer to equilibrium in the same time.

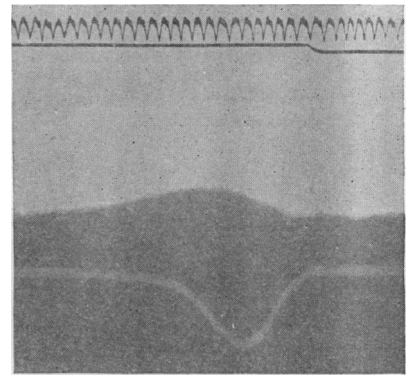

Fig. 4. Manganese dioxide electrode positive, compensated. Increase in positive charge at manganese dioxide electrode beginning 0·010″ after beginning of the shortening of the muscle. Time marker 0·01″.

The considerations which led to the selection of the manganese dioxide electrode are given below in order to indicate the points necessary for the selection of a better electrode.

1. The electrode must be independent of the pressure of oxygen as we do not yet know the variations in oxygen pressure during a muscle contraction. This excludes any form of oxygen or hydrogen electrode with either platinum or iridium. An oxygen electrode would show changes dependent on the use of oxygen by the muscle. A hydrogen electrode if saturated with hydrogen would remove oxygen from the tissues in contact with it so that the conditions of contraction would be abnormal.

Therefore an electrode of the second order containing an insoluble oxide is indicated. This is in equilibrium with the saturated solution of the corresponding hydroxide. The product of the concentration of the metallic and hydroxyl ions is constant and depends on the amount of the hydroxide in solution. Therefore a change in concentration of hydroxyl ions must be accompanied by the reciprocal change in the concentration of metallic ions and must cause a change of potential at the electrode. A decrease in hydroxyl ions will cause the electrode to become more positive.

2. The electrode must not contain anything which exercises a toxic effect on the tissue. Therefore electrodes such as those containing mercuric oxide are inadmissible (3).

3. The electrode must not form an insoluble salt with anything in the tissues. If it

does so the concentration of metallic ions will be so reduced that the product of the concentrations of metallic and hydroxyl ions will be less than that in equilibrium with a saturated solution of the hydroxide. That is the oxide would dissolve and the electrode become useless. Hence silver oxide which would form an insoluble chloride cannot be used.

4. The electrode must be so applied that it will not slip over the surface of the muscle as this would give rise to differences of potential. The movement of the muscle can be minimised by the use of an isometric or tension lever and the electrode should be applied in the form of a thin wire so that it will easily follow the slight movements of the muscle.

The electrical changes have been recorded by photographing the movements of the mercury column of a capillary electrometer (Fig. 2). The form of electrometer *E* was that designed by Dr Keith Lucas[1] for the stage of a microscope and the photographic plate was moved by a Lucas oil-piston (8). The movement of the muscle was recorded on the same plate by the projection of a beam of light from a mirror attached to the axis of the tension lever *T*. The mirror was placed in the same vertical plane as the capillary electrometer so that the spot of light was recorded at *M* on the shadow cast by the mercury column vertically under the record of the height of the mercury at *N*.

The tension lever consisted of a piece of piano wire clamped at the two ends, with a lever brazed on to it midway between the two clamps.

In front of the camera slit *Q* two signals were placed, so that their shadows were recorded on the portion of the plate unshaded by the mercury in the capillary. One of these *O* was placed in the primary circuit of the induction coil and its movement indicated the time at which the muscle was stimulated by a break induction shock. The second signal *P* was a time marker: in some experiments it was a magnetic signal placed in series with a tuning fork giving one hundred vibrations per second and in others it was a clock-work time marker recording fifths of a second.

### *The meaning of the electrical change.*

Galeotti attempted to show an increase in acidity of the beating heart but he used hydrogen electrodes, hence as pointed out above, the tissues may not have been in a normal condition. His results were inconclusive as he found a negative, instead of a positive, charge during contraction. He found however that an indifferent electrode placed on the same portion of the heart gave a greater negative potential. The decrease in the negative potential, he ascribed to the increase in hydrogen ions at the hydrogen electrode (6).

[1] I wish to thank Dr Lucas for help and advice in fitting up the electrometer.

In the present experiments the electrodes were placed exactly opposite each other on a sartorius muscle. Preliminary experiments showed that indifferent electrodes in the same position gave no change of potential on stimulation (10). Further the curves show that the electrical change occurs at a different period of the contraction from the ordinary electrical change. A positive charge at the manganese dioxide electrode is equivalent to a negative charge at the calomel electrode. Since the record, as just mentioned, cannot be due to the ordinary negative change of muscle, a negative charge at the calomel electrode could be caused only by the presence of a negative ion with much greater rate of movement than the positive ions in the solution. The only negative ion that moves more rapidly than most positive ions is the hydroxyl ion and that would be much more effective in making the manganese dioxide electrode negative, hence an increase in hydroxyl ions cannot be the cause of the electrical charge. Therefore the positivity of the manganese dioxide electrode is not due to a negative charge at the calomel electrode.

As described above an increase in acidity should cause a positive charge at the manganese dioxide electrode. The manganese dioxide electrode was connected to the capillary and the movement of the mercury indicated that this electrode became more positive when the muscle contracted, thus indicating an increase in acidity; probably due to the liberation of lactic acid by the muscle during contraction.

*The stage of the contraction at which the acid is produced.*

The photographs show that the movement of the capillary begins soon after the beginning of the contraction. In those cases in which the movement of the mercury is not very great the mercury returns as the muscle relaxes, possibly indicating the removal of acid. When the movement of the mercury is very marked the mercury does not return so quickly. The difference in extent is probably due to the variability of the electrode (see p. 383) and it is probable that when the charge is great the delay in the return of the mercury is explained by the following considerations.

The acid diffuses from the muscle to the surface but the rate of contraction does not allow equilibrium to be attained. Thus it is possible that the amount of acid, in the muscle, may have reached its maximum and be decreasing but there may still be a higher concentration of acid in the muscle than on the surface, so that the acid may be still increasing at the electrode. In the same manner the electrode has

not time to reach equilibrium so that the potential may still be increasing although the acidity at the electrode may have passed its maximum.

The latent period of the shortening of the muscle and the latent period of the movement of the mercury have been measured in a series of experiments and the results are given below.

TABLE I. *Difference in time, in seconds, between beginning of muscle shortening and of movement of mercury in the capillary.*

| | |
|---|---|
| 0·005 | 0·013 (Fig. 3) |
| 0·013 | 0·018 |
| −0·003 | 0·010 (Fig. 4) |
| −0·001 | 0·016 |
| 0·015 | 0·007 |
| 0·005 | 0·008 |
| 0·012 | 0·008 |
| 0·010 | 0·015 |
| 0·010 | |
| Average | 0·0095 ± 0·0009 |

Although the movement of the capillary occurs after the commencement of the shortening of the muscle, the results are in favour of the acid development preceding the muscular contraction. The average delay was 0·0095 ± 0·0009 of a second, but we would, of course, be justified in taking the least difference if we could be certain that the individual observation were correct.

We must remember that there are several factors concerned in the delay of the electrometer record. The acid is produced in the muscle fibre. It must pass through the sarcolemma, and diffuse through any connective tissue between the muscle fibre and the electrode. After the acid reaches the electrode it must alter the equilibrium before the potential can be altered and finally the difference of potential must overcome the inertia of the mercury column.

The hypothesis relating the muscular contraction to acid development I have discussed elsewhere (11). I have assumed that the lactic acid combines with the protein of muscle to form an ionising salt. The protein ion with its positive charge cannot diffuse whilst the lactic ion with its negative charge can diffuse. Thus the ordinary electrical change in muscle would be produced. In this case the time of the ordinary electrical change indicates the time of the formation of the lactic acid salt of the protein. As the amount of any diffusion must be very slight the electrical charge is communicated to the electrodes immediately.

A salt of a weak base with a moderately strong acid is hydrolysed in solution with the formation of some free acid and a rise in the concentration of hydrogen ions. In the case of muscle the amount of lactic acid free to diffuse out is the small amount in equilibrium with the protein salt and not the total concentration of lactic acid.

The conditions of such "membrane equilibria" have been discussed by Donnan (2) but I cannot suggest any time interval for the process.

The length of time for the acid to diffuse from the muscle fibre to the electrode was estimated as follows. Sections of sartorius muscle were stained with van Gieson's stain and the thickness of the connective tissue measured by means of a micrometer eyepiece which had been standardised against a slide ruled in hundredths of a millimeter. On the superficial surface of the muscle there was a compact layer of connective tissue which was nowhere less than three micra in thickness. The deeper surface of the muscle had looser connective tissue and the muscle fibres beneath this connective tissue were not so evenly packed, hence the measurements were not so definite. A measurement of the compact layer of connective tissue on the superficial surface would be a fair estimate for the distance through which the acid must diffuse.

In the paper just referred to (1) the length of time for the diffusion of lactic acid was calculated to be 0·0004 of a second for a distance of one micron. The length of time is proportional to the square of the distance.

Therefore the time interval would be 0·008 of a second. This calculation assumes that the strength of acid is 0·01 normal and that sufficient acid diffuses to cause a change in colour of an indicator such as neutral red. In a membrane equilibrium the strength of acid would be much less than that actually found in the muscle by analysis and the time for diffusion should, therefore, be longer as the difference of concentration would be less.

We therefore see that most of the delay may be referred to the diffusion of acid from the nearest muscle fibre to the electrode.

The potential at the electrode is not an equilibrium condition such as that used in measuring ionic concentrations in the usual way, but it represents the changes at the electrode as it fails to follow the rapid changes in its surroundings. The hydrogen ion concentration is increasing as the acid diffuses from the muscle to the electrode and the potential is also increasing. The electrode would probably approach equilibrium logarithmically, and thus the commencement of the electrical change would begin very rapidly. The movement of the mercury in response to the difference of potential is also a logarithmic relation.

The following method partly suggested by Mr A. V. Hill was used to estimate the delay of the combined electrode and electrometer in recording the increase in acidity at the electrode. The wick of the calomel electrode was suspended transversely in front of the camera slit. On it was placed a piece of blotting paper moistened with Ringer solution and the manganese dioxide electrode was placed on top of the blotting paper exactly opposite to the slit. A tube containing 0·01 normal lactic acid was suspended above the electrode and the movement of the driving piston released a trigger so that the lactic acid was forced through a drawn out point in front of the camera slit.

The interval of time between the arrival of the shadow of the first drop of acid at the shadow of the electrode and the beginning of the movement of the mercury in the capillary electrometer was found to be 0·005 of a second[1]. If we add this time interval to the time calculated for the diffusion of acid from the muscle to the electrode (0·008″) we obtain 0·013 of a second which is more than the 0·0095 of a second found for the difference between the shortening of muscle and the movement of the mercury in the capillary.

*Summation of contractions.*

If the contraction of muscle is due to the production of acid and the acid is removed with the using up of oxygen we can suggest the following mechanism. Stimulation is followed by the production of acid, and as soon as the acid is produced oxidation commences. The contraction begins, but before complete contraction occurs the acid has so decreased that the relaxation commences. If by repeated stimulation the acid can be maintained, the process of contraction can proceed further, that is the height of contraction will be greater (9).

This state of affairs is shown by the electrode as there is a diffusion of acid to the electrode when the acid is in excess in the muscle and a diffusion of acid away from the electrode as the acid is removed by the muscle. As already mentioned it is only in the experiments in which the excursion of the mercury in the electrometer is slight that the inertia of the method allows the return to correspond in time with the muscle contraction.

With repeated contractions the variations of hydrogen ion concentration at the electrode will tend to disappear before the variations in

[1] The addition of a drop of 0·01 normal lactic acid should give a shorter latent period than does the gradual increase in acid at the electrode as acid diffuses from the muscle to the electrode.

acidity in the muscle. On p. 383 it was pointed out that the concentration of acid on the surface of muscle does not rise as high as in the muscle. Therefore, even if the acid in the muscle is reduced to the level of resting muscle, the diffusion of acid away from the electrode will be slower than its diffusion to the electrode as the difference in concentration will be less. A fresh development of acid in the muscle will therefore tend to cause a summation of acid at the electrode before complete summation of acid occurs in the muscle. Similar arguments show that the potential at the electrode will tend to fuse before the acid concentration at the electrode becomes uniform. The inertia of the electrometer will also tend to smooth away slight differences in potential especially if they follow each other rapidly.

From these considerations we may expect to find that fusion of the potential due to acid will occur with a slower rate of stimulation than is necessary to produce complete tetanus, and this is in fact what the records show.

The muscle was stimulated by a series of stimuli caused by a vibrating spring in the primary circuit making and breaking contact with a mercury pool. The movement of the driving piston opened a short circuit in the secondary circuit. The reproductions show the contacts made by the vibrating spring and the contractions of the muscle indicate when the short circuit is opened. In a few experiments the time marking was in hundredths of a second, but in most it was in fifths.

All stages of summation, from a series of separate contractions with separate electrical changes to an almost complete tetanus with complete

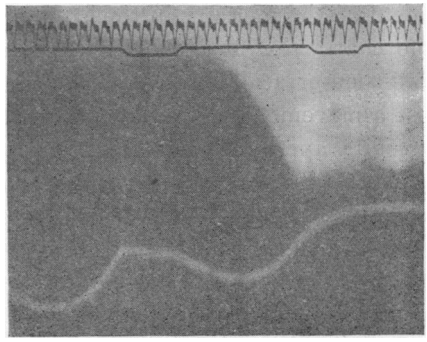

Fig. 5. Manganese dioxide electrode positive, compensated. Two partially fused contractions with partially summed increases in positive charge at manganese dioxide electrode. Time marker 0·01″.

fusion of electrical changes, have been obtained. Figs. 5 and 6 illustrate two of these stages. The figures suggest that the acid production in muscle is associated with contraction, increasing during the mechanical shortening and decreasing during relaxation. With

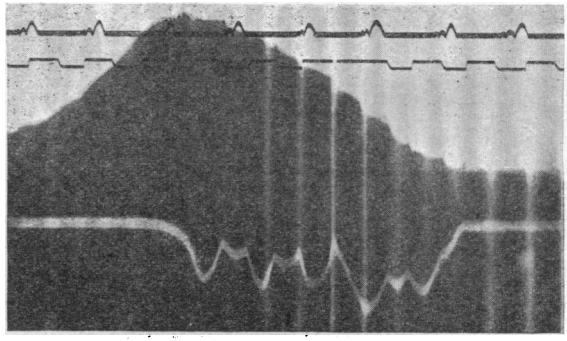

Fig. 6. Manganese dioxide electrode positive, compensated. Partial fusion of contractions with almost complete fusion of positive charges at manganese dioxide electrode. Time marker 0·2″.

repeated stimuli the acidity at the surface of the muscle seems greater than with single stimuli and its summation occurs with a slower rate of stimulation than is necessary to cause complete tetanus.

## SUMMARY.

1. An electro-chemical method is described for recording the increase in acidity when a muscle contracts.

2. The movement of the mercury in the capillary electrometer was found to follow the contraction by ·0095 of a second on an average. The time for the diffusion of acid is calculated to be ·008 sec. and that for the acid to cause a movement of the mercury in the capillary to be ·005 sec. These results appear to me to favour the view that the increase in acidity is the cause of the shortening of muscle.

3. Repeated stimuli lead to a fusion of electrometer curves due to increase in acidity before fusion of contractions occurs.

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